Field-Based Estimates of Heritability and Genetic Correlations in Hop

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ABSTRACT

Hop (*Humulus lupulus* L. var. *lupulus*) is grown worldwide for the production of the dried female inflorescence (strobulus), or cones, used principally for the bittering and flavoring of beer. Information is scant on the inheritance of traits of economic importance in hop, and present knowledge is based on historical data rather than designed experimental investigation. The objective of this study was to estimate the heritability of and genetic correlation among six traits: yield (YLD), α-acid (ALP) concentration, β-acid (BET) concentration, cohumulone (COH) percentage, colupulone (COL) percentage, and xanthohumol (XAN) concentration. Twenty-five full-sib families were developed by crossing five randomly chosen females and five randomly chosen males in a North Carolina Design II mating design. Plants were transplanted into the field in a randomized complete block (RCB) design with four replicates. Data were recorded for two years. Heritabilities for all traits were moderate to high using variance components estimated from males. With the exception of heritability estimates for YLD and ALP, all other traits were not significantly different from zero using female variance components as estimators. Pooled estimates of heritability yielded more reasonable estimates with lowest heritability for BET ($h^2 = 0.57 \pm 0.19$) and highest for COL ($h^2 = 0.89 \pm 0.02$). Pooled estimates of genetic correlations ranged from $r = 0.28$ (ALP and YLD) to $r = 0.92$ (YLD and XAN). Finally, correlations between coefficients of coancestry (COA) between pairs and their respective mean offspring data were significant for ALP, COL, and XAN suggesting that for these traits at least, COA values may be predictive of potential heterosis. On the basis of these data, selection for COL, ALP, and YLD would be successful using simple selection protocols such as phenotypic recurrent or mass selection. The likelihood of success when selecting for BET and XAN would be low, thus requiring one of the genotypic recurrent selection techniques. Selection against COH (a negative factor in brewing) appears problematic because of positive correlations with all other traits. The information presented in this study is the first published record of field-based estimates of narrow-sense heritability and genetic correlations in hop and will aid hop breeders working with these traits.

T HE HOP PLANT is a bine-climbing perennial plant that grows principally within the latitudes of 35 to 55° both north and south of the equator (Neve, 1991). *Humulus* species are dioecious and, as such, are one of the few cultivated genera that have true sex chromosomes. The harvested product from hop is the unfertilized female inflorescence (strobulus), or cones. Male hop plants are kept solely for breeding purposes and are generally classified as "noxious weeds" in hop-growing regions.

Early selection efforts in hop breeding consisted solely of clonal selection, with most male hop plants rogued out from localities where hops are produced. Only within the last half-century have major efforts been made to utilize male hop lines in breeding programs. Because of the paucity of male hop lines and the insistence by major brewers on using specific established hop varieties, little information has accumulated regarding the genetics of breeding this species, other than early work by Keller and Likens (1955) and work using historical data for estimates of heritability (Henning et al., 1997a, 1997b). Furthermore, the lack of male lines has restricted efforts by researchers to estimate maternal and paternal effects in genetic studies. Initial reports on hop breeding dealt primarily with technique and strategy with little information on the heritabilities of, or genetic correlations between, traits of interest (Haunold, 1980, 1981; Neve, 1991; Roberts et al., 1980). Keller and Likens (1955) and Roberts et al. (1980) both reported on heritability in hops. Unfortunately, both papers reported heritability estimates that were biased upward because of confounding of the additive genetic variance with dominance variance.

Much of the preliminary efforts at breeding hops through cross-fertilization focused primarily on choice of female lines for crossing; the male parent viewed solely as a means to obtain offspring for selection. Initial genetic work by Henning et al. (1997a) demonstrated that significant genetic variation was present in male lines, which could be utilized to choose specific males for crossing purposes on the basis of general combining ability (GCA) and also specific combining ability (SCA). Henning et al. (1997a, 1997b) used historical data to estimate genetic variances, heritabilities, and genetic correlations for several traits. Traits with moderate to high additive genetic variance and heritability were ALP, BET, and hop storability. Heritability estimates for YLD and essential oil content were low. Estimation of environment and genotype × environment (G × E) effects was not reported in these studies because of the use of historical field data that were not replicated across either years or locations. Thus, published narrow-sense heritability estimates are biased upward because of the confounding effects of environment and G × E effects and accurate estimates still are needed to identify appropriate breeding plans and goals in hop.

Greatest genetic gain due to selection on a yearly basis is usually the goal of plant breeders. There are various ways to maximize this in breeding programs. Gain due to selection is defined as:

$$\Delta G = i\sigma_P h^2 = i\sigma_A^2/\sigma_P \qquad [1]$$

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Abbreviations: ALP, α-acid; BET, β-acid; COA, coefficients of coancestry; COH, cohumulone; COL, colupulone; GCA, general combining ability; RCD, randomized complete block; SCA, specific combining ability; XAN, xanthohumol; YLD, yield.

with ΔG defined as the genetic gain due to selection, i defined as the selection intensity, σ_P defined as the phenotypic standard deviation, h^2 defined as the narrow-sense heritability, and σ_A^2 defined as the additive genetic variance. Maximizing any one of these factors, i, σ_A^2, or h^2, will result in a higher genetic gain because of selection. Because h^2 is equivalent to σ_A^2/σ_P^2 it follows that maximizing σ_A^2 relative to σ_P^2 will increase gain because of selection. This is typically done by some means of progeny testing to minimize environmental influences and obtain more accurate additive genetic variance estimates (Comstock and Moll, 1963). Furthermore, choice of both male and female parents with high GCA for specific traits, on the basis of progeny testing, also maximizes σ_A^2 relative to σ_P^2. Increasing i by performing selection over a one-year period, through some means of phenotypic selection rather than spreading selection cycles over several years through progeny testing, should increase gain because of selection per year (Allard, 1960). Likewise, in cases where high amounts of dominance (D) or G × E interactions bias the heritability upward, it would be beneficial to utilize some means of family selection or progeny testing, coupled with multiple locations or years, to better estimate genetic worth (Falconer, 1983). However, if D and/or G × E effects are negligible and the resulting heritability high, then it follows that mass selection or phenotypic recurrent selection would probably result in the highest genetic gain from selection on a yearly basis.

Comstock and Robinson (1952) proposed a mating design (North Carolina Design II) that resulted in male half-sib families and female half-sib families along with *Male × Female* full-sib families. The elegance of this design is that specific components of the resulting analysis of variance (ANOVA) are used to directly test for significant additive and dominant genetic variance, and also test for the significance of environment and G × E interaction. Estimates of additive (V_a) and dominance genetic variance (V_d) components and the resulting estimate of narrow-sense heritability are also obtained by this design through equating specific mean squares in the ANOVA to family covariances such as half-sib covariances and full-sib covariances (Kempthorne, 1957). Additionally, when looking at several traits, one can obtain estimates of genetic correlations among the multiple traits of interest. In most agronomically important plant species, the designation of "male" and "female" is arbitrary and nonessential. Hop is a dioecious species, however, and estimates of genetic variance for males and females are important. Comparing males for general combining ability is a critical factor used in selecting male parents for inclusion into elite germplasm pools. In most cases, female offspring may be selected for elite germplasm pools on the basis of phenotypic expression if heritability for specific traits of interest is high. Thus, the North Carolina Design II provides all the necessary information to pursue the development of an elite hop germplasm pool, and provides information on what breeding methods should be utilized to maximize selection efficiency.

The objective of this study was to determine genetic

variances and heritability estimates, and genetic correlations among six important hop traits: ALP, BET, COH, COL, YLD, and XAN.

MATERIALS AND METHODS

All possible crosses were made between five males (M21262, M21266, M21267, M21338, and M21378) and five females ('Challenger', 'Nugget', 'Omega', 'Orion', and 'Magnum') using a North Carolina Design II mating in 1997 (Table 1). Secondary branches on female hop lines were bagged before burr stage to eliminate fertilization. Once female flowers were receptive to pollination, pollen from a specific male was then added to the enclosed bag and the bag shaken to distribute the pollen inside the bag. These secondary branches were subsequently harvested separately and the seed threshed out. Seeds were then categorized according to cross, treated for dormancy (Haunold, 1980), and then planted in jiffy pots during the spring of 1998. During the month of June 1998 seedlings were transplanted out to the USDA-ARS hop research facility located near Corvallis, OR. Plants were individually spaced on a 2.28 by 2.28 m grid. The experimental design was an RCB design with four replicates and five genotypes per family per replicate (total of 20 genotypes per full-sib family). Average daily temperatures during the growing season ranged from 16 to 27°C with daylength ranging from 13 to 16 h d^{-1}. Soil type was a Chehalis silty clay loam (fine-silty, mixed, superactive, mesic Cumulic Ultic Haploxerolls). Fertilizer (168 kg ha^{-1} urea) and irrigation were applied as necessary and represented treatments as typically performed by Oregon hop producers. Data (ALP, BET, COH, COL, YLD, and XAN) were collected from individual genotypes during the 1999 and 2000 growing seasons with years representing environments. Yield was obtained by means of whole plant harvests using a Wolf Type I (Wolf Anlagen-Technik GmbH & Co. KG, Geisenfeld, Germany) mechanical picking machine. Fresh weight of hop cones per plant (g plant^{-1}) were recorded and transformed by means of multiplying by

Table 1. Hop males and females, along with filial pedigree for each genotype, used in North Carolina Design II cross.

Accession or variety	Parents	Pedigree
Males		
M21262	62013 × 21110M	Comet†/4/Brewers Gold//Early Grape/unknown male/3/Zattler Seedling‡
M21266	62013 × 21110M	Comet//Bullion/Zattler Seedling
M21267	62013 × 21110M	Comet//Bullion/Zattler Seedling
M21338	64107 × 21111M	Northern Brewer//Bullion/Zattler Seedling
M21378	56013 × 21136M	Cascade/4/Brewers Gold//Early Grape/unknown male/3/Zattler Seedling
Females		
Challenger	17/54/2 × 1/61/57§	Zattler Female/Unk. Male//Unk. Male/3/'Wye 22/56'§/Northern Brewer
Nugget	65009 × 63015M	Brew. Gold//Early Grape/Unk. Male/4/Brew. Gold/3/E. Kent Golding//Bavarian/Unk. Male
Omega	4/64/25 × Unknown	Challenger/Unk. Male
Orion	21227 × 70/10/15¶	Northern Brewer/German male//German male
Magnum	21182 × 75/5/3¶	Galena#/German male

† Comet resulted from the cross Sunshine/Utah 524-2 (wild American hop from Utah).
‡ Experimental line developed at Huell Hop Research Center, Huell, Germany.
§ Wye College (Wye, England) accession numbers.
¶ Huell Hop Research Center designation, Huell, Germany.
# Galena resulted from the cross Brewers Gold/unknown male.

a predetermined factor (0.4777) into kilograms dry weight per hectare for yield analyses. Hop cones were subsequently dried to approximately 8% moisture for all chemical analyses and stored at 5°C until processed for analysis. Chemical analyses were performed using HPLC according to industry standard methods (American Society of Brewing Chemists, 1992).

Data were analyzed and mean squares equated to genetic expectations according to the North Carolina Design II model reported by Comstock and Robinson (1952) and further delineated by Hallauer and Miranda (1981). This model utilizes a set of male genotypes and an independent set of female genotypes crossed in all possible combinations. These crosses result in a set of full-sib families and half-sib families. The half-sib families are further subdivided into paternal half-sib families and maternal half-sib families. The analysis of variance from this design results in variance component estimates that are directly equated with genetic covariances that represent estimates of additive and dominance genetic variance (Table 2). The experiment was analyzed as a mixed model with replications considered as a fixed variable while male and female accessions and years were considered as random variables. The interactions Year × Female × Male and Year × Replication were not significant so they were pooled into the appropriate error term.

All statistical analyses and the generation of matrices were performed using SAS for Windows Release 7.0 (SAS Institute, Cary, NC). Manipulation of matrices and calculations of heritabilities and genetic correlations were accomplished using MS Excel 97 (Microsoft Inc, Bellevue, WA). Heritabilities, genetic correlations and standard errors for heritability estimates were calculated using formulas reported by Hallauer and Miranda (1981) using pooled male and female variances. The equations used to estimate heritability and the standard errors of heritability estimates were as follows:

$$h^2 = \frac{2(\sigma_m^2 + \sigma_f^2)}{\sigma_e^2/(rfy) + 2(\sigma_{my}^2/y + \sigma_{fy}^2/y) + \sigma_{mf}^2/f + 2(\sigma_f^2 + \sigma_m^2)} \quad [2]$$

$$SE(h^2) = \frac{SE[2(\sigma_m^2 + \sigma_f^2)]}{\sigma_e^2/(rfy) + 2(\sigma_{my}^2/y + \sigma_{fy}^2/y) + \sigma_{mf}^2/f + 2(\sigma_f^2 + \sigma_{fm}^2)} \quad [3]$$

with e representing experimental error, m representing males, f representing females, y representing years, and r representing

Table 2. Analysis of variance showing expected mean squares and estimated genetic component for each factor.

Source	df	Expected mean squares†	Estimated genetic or environment component‡
Rep	3		
Year	1	$\sigma_e^2 + rf\sigma_{ym}^2 + rm\sigma_{yf}^2 + rmf\sigma_y^2$	$\sigma_y^2 = V_y$
Female × Year	4	$\sigma_e^2 + rm\sigma_{yf}^2$	$\sigma_{yf}^2 = V_{y \times f}$
Male × Year	4	$\sigma_e^2 + rf\sigma_{ym}^2$	$\sigma_{ym}^2 = V_{y \times m}$
Female	4	$\sigma_e^2 + rm\sigma_{yf}^2 + yr\sigma_{fm}^2 + yrm\sigma_f^2$	$\sigma_f^2 = V_f = 0.25 V_a$
Male	4	$\sigma_e^2 + rf\sigma_{ym}^2 + yr\sigma_{fm}^2 + yrf\sigma_m^2$	$\sigma_m^2 = V_m = 0.25 V_a$
Female × Male	16	$\sigma_e^2 + yr\sigma_{fm}^2$	$\sigma_{fm}^2 = V_{f \times m} = 0.25 V_d$

† f, female; m, male; r, replication; y, year.
‡ V_a, additive genetic variance; V_d, dominance genetic variance.

replications. Calculations of genetic correlations were performed using the following equation

$$r_g = \frac{Cov(ij)}{\sqrt{[\sigma^2(i)\sigma^2(j)]}} \quad [4]$$

with r_g representing the genetic correlation between two traits, $Cov(ij)$ representing the average (averaged across male and female covariance estimates) additive genetic covariance between traits i and j, $\sigma^2(i)$ representing the average (averaged across male and female variance estimates) additive genetic variance for trait i, and $\sigma^2(j)$ representing the average additive genetic variance for trait j. Coefficients of coancestry for each male–female pair were calculated using the program PEDI-GREE VIEWER (Kinghorn and Kinghorn, 2001), which bases calculations of COA on the method reported by Kempthorne (1957). Finally, dendrograms derived to explain relationships between males and females were calculated and produced by use of Systat Ver 10.2 (Systat Software Inc, Richmond, CA).

RESULTS

We observed significant F tests ($P \leq 0.05$) for the Female × Male interaction in all traits except COL (Table 3). Significant differences were observed for both females and males for YLD and ALP. Variance components for males were also significantly different from zero for COH, COL, BET, and XAN while there were no significant differences among females for these traits. We observed only one example of G × E interaction in the effects for XAN. This was observed for Male × Year but not for Female × Year in XAN. Finally, we observed significant differences among years for the expression of YLD, BET, and COL but not for ALP, COH, and XAN.

We observed several points of interest regarding differences in trait expression among full-sib families or crosses. The cross Nugget × M21267 exhibited the highest YLD among families (Table 4). Magnum × M21267 had the highest concentration of ALP among the crosses but was not significantly different from eight other crosses on the basis of Fisher's protected LSD tests. Results for BET were more conclusive with the cross Magnum × M21378 exhibiting the highest concentration, although it was not significantly different in concentration from the cross Challenger × M21267. Four crosses, which were not statistically different from one another, expressed the highest levels of COH among all crosses (Table 5). High COH is typically considered a detriment to brewing. Conversely, high levels of COL are considered desirable for brewing purposes. We observed nine crosses

Table 3. Analysis of variance showing mean squares and significance for yield (YLD), α-acid concentration (ALP), β-acid concentration (BET), cohumulone percentage (COH), colupulone percentage (COL), and xanthohumol concentration (XAN).

Source	df	YLD	ALP	BET	COH	COL	XAN
Rep	3	879 342*	20.54*	1.22	361.45*	215.78*	0.035
Year	1	4 292*	70.32	34.77*	223.16	968.54*	0.351
Female × Year	4	3 219	11.53	1.21	17.04	43.42	0.025
Male × Year	4	3 086	11.91	1.65	27.64	0.737	0.083*
Female	4	878 425*	70.49*	5.60	44.45	67.43	0.062
Male	4	1 139 353*	85.64*	9.88*	742.96*	1217.0*	0.320*
Female × Male	16	299 857*	13.38*	3.08*	54.48*	80.66	0.059*

* Fisher's F test significant at $P \leq 0.05$

Table 4. Crosses between five females and five males, the coefficients of coancestry (COA) for each cross and the means for each cross for yield (YLD), α-acid concentration (ALP), and β-acid concentration (BET). Means with the same letters within a column are not significantly different from one another based on Fisher's Protected LSD with $P \leq 0.05$.

Genetic Cross	COA†	YLD	ALP	BET
		kg ha^{-1}	——— v/v ———	
Challenger × M21262	0.0039	819 d	5.53 efghi	1.93 cdefg
Challenger × M21266	0.0020	722.1 d	5.19 cdefg	1.83 bcde
Challenger × M21267	0.0020	660.8 cd	6.62 ijk	2.36 gh
Challenger × M21338	0.0645	414.0 a	3.26 a	1.13 a
Challenger × M21378	0.0039	744.8 d	3.69 ab	1.31 a
Nugget × M21262	0.0859	858.7 d	4.85 abcdefg	1.94 bcdefg
Nugget × M21266	0.0195	787.0 d	4.98 abcdefg	1.54 abc
Nugget × M21267	0.0195	1048.9 e	5.01 abcdefg	1.55 abc
Nugget × M21338	0.0586	651.0 cd	4.30 abc	1.47 ab
Nugget × M21378	0.0859	682.2 d	4.14 ab	2.13 efg
Omega × M21262	0.0020	677.2 d	6.11 fghijk	1.97 cdefg
Omega × M21266	0.0010	402.2 a	4.71 abcde	1.25 a
Omega × M21267	0.0010	843.5 d	6.24 ghijk	1.56 abc
Omega × M21338	0.0322	507.5 abcd	5.59 efghi	1.54 abc
Omega × M21378	0.0020	466.7 abcd	4.79 abcdef	1.60 abcde
Magnum × M21262	0.0234	637.5 cd	6.32 ghijk	2.13 defg
Magnum × M21266	0.0156	666.9 c	7.06 jk	1.86 bcdefg
Magnum × M21267	0.0156	683.5 cd	7.17 k	2.27 fg
Magnum × M21338	0.0313	644.0 cd	5.32 defgh	1.53 abc
Magnum × M21378	0.0234	434.4 ab	6.45 hijk	2.82 h
Orion × M21262	0.0039	504.0 abcd	5.96 efghij	1.95 cdefg
Orion × M21266	0.0020	610.9 bcd	6.79 ijk	1.87 bcdef
Orion × M21267	0.0020	672.0 cd	7.14 jk	2.17 efg
Orion × M21338	0.0645	479.1 abc	3.69 ab	1.13 a
Orion × M21378	0.0039	522.7 abcd	5.67 efghij	1.94 cdefg

† Coefficient of coancestry; relationship value calculated as reported by Kempthorne (1957).

that exhibited high levels of COL. Finally, there were five statistically similar crosses that exhibited high levels of the potential anticancer agent XAN.

Table 5. Crosses between five females and five males, the coefficients of coancestry (COA) for each cross and the means for each cross for cohumulone percentage (COH), colupulone percentage (COL), and xanthohumol concentration (XAN). Means with the same letters within a column are not significantly different from one another on the basis of Fisher's Protected LSD with $P \leq 0.05$.

Genetic cross	COA†	COH	COL	XAN
		——— v/v ———		mg mL^{-1}
Challenger × M21262	0.0039	33.94 ghi	54.66 cdef	0.191 defg
Challenger × M21266	0.0020	31.06 def	52.81 bcd	0.214 efgh
Challenger × M21267	0.0020	29.31 bcde	52.63 bcd	0.314 j
Challenger × M21338	0.0645	26.67 ab	45.77 a	0.089 a
Challenger × M21378	0.0039	32.00 efg	53.66 bcde	0.097 ab
Nugget × M21262	0.0859	34.92 ghi	54.50 cdef	0.154 abcde
Nugget × M21266	0.0195	30.57 def	53.77 bcde	0.212 efgh
Nugget × M21267	0.0195	32.58 fgh	56.78 ef	0.235 efghi
Nugget × M21338	0.0586	29.31 bcde	48.25 a	0.157 bcde
Nugget × M21378	0.0859	30.90 cdef	54.60 bcdef	0.175 cde
Omega × M21262	0.0020	36.11 i	57.56 ef	0.203 defgh
Omega × M21266	0.0010	28.34 abcd	50.97 bc	0.168 cde
Omega × M21267	0.0010	31.13 def	55.50 def	0.186 def
Omega × M21338	0.0322	28.70 abcd	51.40 bc	0.138 abcd
Omega × M21378	0.0020	28.71 abcd	51.06 bc	0.109 abc
Magnum × M21262	0.0234	32.73 fgh	54.88 cdef	0.185 defg
Magnum × M21266	0.0156	30.33 bcdef	52.76 bcd	0.263 ghij
Magnum × M21267	0.0156	31.97 efg	53.31 bcd	0.243 efghi
Magnum × M21338	0.0313	28.88 abcd	48.50 a	0.139 abcd
Magnum × M21378	0.0234	27.65 abc	50.52 b	0.281 ij
Orion × M21262	0.0039	35.71 hi	58.58 f	0.197 defg
Orion × M21266	0.0020	28.88 abcd	52.29 bcd	0.251 fghij
Orion × M21267	0.0020	30.90 def	55.55 def	0.274 hij
Orion × M21338	0.0645	25.88 a	45.06 a	0.107 abc
Orion × M21378	0.0039	28.67 abcde	53.28 bcde	0.162 bcde

† Coefficient of coancestry; relationship value calculated as reported by Kempthorne (1957).

Crosses made with Nugget produced offspring with the highest YLD that were significantly higher than each of the other maternal half-sib families (Table 6). Conversely, while Nugget produced offspring that exhibited the highest YLD, the lowest levels of ALP were seen in offspring produced by this variety. The maternal half-sib family generated from Magnum exhibited the highest levels of ALP among all the varieties tested. There were no significant differences among maternal half-sib families for the levels of BET, COH, COL, and XAN observed in the offspring.

The paternal half-sib families produced by M21267 and M21262 generated offspring that had the highest YLD (Table 6). Offspring from the male M21267 produced the highest average ALP among the males tested. The three paternal families produced by M21262, M21267, and M21378 exhibited the highest levels of BET. Male M21338 produced offspring that possessed lowest COH concentrations while also producing offspring that had the lowest COL among all lines. Highest COL was observed for offspring from males M21262 and M21267. Finally, the male paternal families obtained from M21266 and M21267 both exhibited the highest levels of XAN.

To determine whether COA estimates may be used as predictors for heterosis in hops, we calculated COA values for all crosses, and then calculated correlations between COA values and the average values for YLD, ALP, BET, COH, COL, and XAN (Table 7). As COA values increase (males and females become more related by descent), we should see evidence of inbreeding taking effect such as reduced YLD or reduced ALP. We observed significant negative correlations between COA and ALP, COL, and XAN.

Heritability estimates for females were not calculable for BET, COH, COL, and XAN because of insignificant variation among female half-sib families. As a result, we calculated pooled heritabilities using pooled variances from males and females. Narrow-sense heritabilities based on pooled estimates of male and female variance com-

Table 6. Maternal and paternal half-sib family averages for yield (YLD), α-acid concentration (ALP), β-acid concentration (BET), cohumulone percentage (COH), colupulone percentage (COL), and xanthohumol concentration (XAN). Means within a column and subheading of male or female that have similar letters are not significantly different from one another on the basis of Fisher's LSD test with $P \leq 0.05$.

Family	YLD	ALP	BET	COH	COL	XAN
	kg ha^{-1}	——————— v/v ———————				
		Maternal family				
Challenger	668.4 b	4.85 a	1.71 a	30.6 a	51.9 a	0.18 a
Nugget	801.0 c	4.63 a	1.73 a	31.5 a	53.5 a	0.18 a
Omega	586.2 a	5.46 b	1.58 a	30.5 a	53.2 a	0.16 a
Magnum	609.8 ab	6.45 c	2.13 a	30.2 a	51.9 a	0.22 a
Orion	556.3 a	5.80 b	1.81 a	29.8 a	52.9 a	0.20 a
		Paternal family				
M21262	697.4 cd	5.74 b	1.99 c	34.6 d	56.0 c	0.19 b
M21266	634.4 bc	5.70 b	1.66 b	29.7 b	52.5 b	0.22 c
M21267	783.2 d	6.42 c	1.99 c	31.1 c	54.7 c	0.25 c
M21338	533.9 a	4.40 a	1.36 a	27.8 a	47.8 a	0.12 a
M21378	572.9 ab	4.93 a	1.96 c	29.5 b	52.5 b	0.16 b

Table 7. Pearson correlation coefficients between coefficient of coancestry (COA) values calculated for specific crosses, as listed in Tables 4 and 5, and the crosses' respective average values for six indicators of economic value: yield (YLD), α-acid concentration (ALP), β-acid concentration (BET), cohumulone percentage (COH), colupulone percentage (COL), and xanthohumol concentration (XAN).

TRAIT	COA
	r
YLD	−0.020
ALP	−0.554**
BET	−0.174
COH	−0.172
COL	−0.422*
XAN	−0.405*

* Significant at the $P \leq 0.05$ level.
** Significant at the $P \leq 0.01$ level.

ponents were highest for COL and COH, intermediate for ALP and YLD, and lowest for both XAN and BET (Table 8).

Pooled genetic correlations among the five traits illustrated several genetic covariation results that should be addressed. Ten positive correlations were measured; the remaining five were not significant (Table 8).

DISCUSSION

Yield is an important component for any breeding program in any crop and, as such, should be one of the first factors considered when setting out to study the heritabilities of specific factors of economic importance. The other factors, ALP, BET, COH, COL, and XAN, are all chemical components that factor into the flavoring quality of a specific hop variety or present opportunities for pharmaceutical industries. High concentrations of the bittering acids ALP and BET are desired as higher concentrations of these two components reduce costs for brewing. Low concentrations of COH are desired by many brewers as this particular compound is viewed as a negative flavoring component. Higher concentrations of COL are considered a positive goal for most breeding programs. Finally, recent findings on XAN suggest the potential of this compound in fighting certain human cancers (Henderson et al., 2000; Miranda et al., 1999, 2000). Thus, as the first report on field-based heritabilities of important characters in hops, these factors were considered the most important to study.

Table 8. Pooled genetic correlation (r_g) and narrow-sense heritability (h^2_{pooled}) for yield (YLD), cohumulone percentage (COH), colupulone percentage (COL), α-acid concentration (ALP), β-acid concentrations (BET), and xanthohumol concentrations (XAN) calculated from the genetic variance estimates for five female and five male hop accessions crossed in a North Carolina II mating design.

TRAIT	Heritability	YLD	COH	COL	ALP	BET	XAN
	h^2_{pooled}			r_g			
YLD	0.71 ± 0.001						
COH	0.87 ± 0.03	0.636*					
COL	0.89 ± 0.02	0.685*	0.881**				
ALP	0.76 ± 0.06	0.282	0.385	0.592			
BET	0.57 ± 0.19	0.729*	0.775**	0.874**	0.714*		
XAN	0.60 ± 1.14	0.921**	0.509	0.759*	0.864**	0.588	

* Significant at the $P \leq 0.05$ level.
** Significant at the $P \leq 0.01$ level.
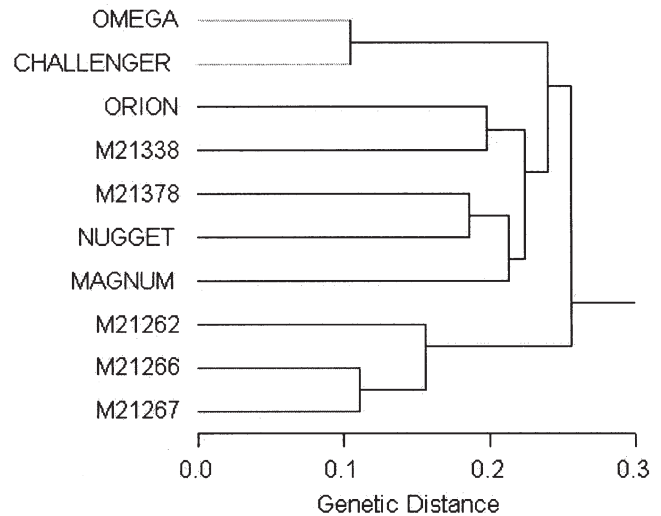
Fig. 1. Genetic distances (GD) based on pedigree analysis among five females and five male hop accessions used in North Carolina Design II crossing scheme. Clustering was accomplished using Ward's Clustering with distance values based on a modification of coefficient of coancestry (COA) with GD = 1 − 2COA.

This study utilized only "years" as the environmental variable. Further evaluations incorporating different locations in addition to years would be required to accurately estimate G × E effects. Furthermore, additional work with a greater number of accessions is warranted before conclusively determining whether multiple environments are needed. If multiple environments prove unnecessary, this could result in significant savings to breeding programs by negating the need for multiple site analysis to accurately estimate the breeding value of a particular line.

The males and females were randomly selected from released cultivars and USDA breeding material. Unfortunately, many of the currently available hop varieties (females) are related to one another because they descend from a few common ancestors that were highly prized by early breweries for their brewing properties. The ancestral relationship among males and females chosen for this study illustrates the lack of variation among female varieties (Fig. 1). According to pedigree analysis, the accessions fall into only two distinct groups with the females and two of the males (M21338 and M21378) clustered into one group. The remaining three males (M21262, M21266, and M21267) comprise the second group. This divergent group of three males has significant contributions from wild American germplasm in their ancestry. As already mentioned, the choice of female and male lines used in this study was accomplished by random drawing of individuals making up a subset of all the genotypes present in the USDA-ARS hop germplasm pool. It is interesting to note the close genetic similarity between the varieties Omega and Challenger. Challenger is the mother of Omega. Orion is a granddaughter of the variety 'Northern Brewer' just as Challenger is, though through a different mother than the mother of Orion. In addition, all of the females have as a common ancestor, the variety 'Brewers Gold'. These close relationships among females may explain the lack

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of genetic variation among females observed in this study while the two groups of males may explain the significant variation present among males for many of the traits.

Significant interactions for Female × Male were observed in this study for all traits except COL. Female × Male interaction factors represent the deviation from the mean because of dominance variance (Comstock and Robinson, 1952). Thus, dominance played a significant role in determining the expression of specific traits. Breeding for the expression of dominance is not accomplished through individual, mass, or recurrent selection, but by the selection of parental pairs that express higher than average traits when crossed—in short, the specific combining ability for known pairs. Furthermore, in theory, the greater the genetic distance between two individuals the greater the dominance deviation or heterosis that will be observed (Falconer, 1983). Looking at the relationship between COA and the expression of three of the traits examined (ALP, COL, and XAN) demonstrated significant negative genetic correlations—which suggests the possibility of using COA estimates as predictors of heterosis (Table 7). In this case, two parents having low COA would be highly divergent from one another and expected to have higher than average expression of specific traits. Pairs of parents having a high COA are related to a great extent and would be expected to have reduced expression of traits because of inbreeding depression. These results are the first demonstration of predicting heterosis in hops by means of genetic similarity among parents.

The selection of parents for any breeding effort is made easier by collection and utilization of data from the offspring that a particular male or female generates. In this study, the variety Nugget was the best female parent for YLD, while Magnum was the best female parent for ALP. The male lines M21262 and M21267 both exhibited offspring that had higher than average yields. Magnum and one of the two high-yielding males, M21262 or M21267, would likely produce high-yielding, high ALP offspring. Certainly, the moderately high heritabilities seen for both traits would lend support for success in attaining higher YLD and ALP when choosing these parents.

Narrow-sense heritabilities reported here differ somewhat from those published previously (Henning et al., 1997a). On the basis of standard errors associated with the estimation of heritabilities in both Henning et al. (1997a) and this work, there appears to be no differences in values. We observed differences in genetic correlations among traits from prior publications concerning the relationships between YLD, ALP, and BET. Henning et al. (1997a) observed negative correlations between ALP and BET, while we observed a positive correlation (Table 6). Furthermore, Henning et al. (1997a) observed a significant negative correlation between YLD and ALP, while our study did not find any correlation. The reason for these differences may lie in the structure of the populations used to estimate genetic components of variance. Henning et al. (1997a) used data from historical archives that were taken over a period of 25 yr. Records during this period were maintained only on selected individuals rather than random offspring. Historically, USDA-ARS aroma varieties were developed by selecting against high ALP and selecting for high YLD. Aroma varieties typically have low to moderate ALP (~5 to 7% v/v). Thus, those varieties possessing high YLD, but low to moderate ALP, were selected and maintained for data collection, which would explain the negative correlation between these two traits. Historical attempts at selecting for aroma varieties presumed that selection for equal but low levels of both ALP and BET was most desirable for brewing purposes. Perhaps the historical selection for YLD while selecting for reduced ALP had an indirect effect on increasing BET. Certainly, the significant ($P < 0.01$) positive genetic correlation between BET and YLD in the Henning et al. (1997a) paper would suggest such a response. Regardless, we observed a positive genetic correlation between ALP and BET, and as these results were obtained using random offspring and a less confounded mating design (North Carolina Design II rather than Design I), the results reported herein are likely more representative of the actual genetics of these traits in hop.

Selection for YLD should result in concomitant increases in BET, COH, COL, and XAN. Simultaneously, selection for BET should result in concomitant increases in YLD, ALP, COH, and COL. The compound COH is viewed by many in the brewing industry as a negative component and selection should be directed against increased levels. Unfortunately, selection for increased BET and YLD should increase levels of COH simultaneously, resulting in a dilemma for the breeder. A break of the linkage between BET and COH, and also between YLD and COH, should be sought in the development of breeding populations before attempting further progress regarding these three traits.

This study is the first published report of field-based estimates of narrow-sense heritabilities, genetic correlations, and estimation of heterosis potential in hop using COA pedigree analyses. Further work needs to be done concerning the heritability of specific essential oils and their genetic relationships to other traits of economic importance. Selection for specific traits such as YLD and ALP are realizable goals on the basis of relatively high heritabilities for these traits. Because of the significant Female × Male interactions, which equates to dominance controlling many of the traits investigated in this study (Table 3), selection for specific parents to produce hop hybrids is another technique that should be performed by hop breeders. Furthermore, verification of heterosis prediction based on molecular tools should be pursued, as molecular measurements for genetic distance between parents should offer a more accurate measure of genetic distance than does COA pedigree analysis. Hop variety development is expensive and the significant dominance present for many of the traits examined here suggests that hop breeding would benefit by having an accurate molecular measure of estimating potential heterosis.

REFERENCES

Allard, R.W. 1960. Principles of plant breeding. John Wiley & Sons Publ., New York.

American Society of Brewing Chemists. 1992. Methods of analysis. 8th ed. The American Society of Brewing Chemists, St. Paul, MN.

Comstock, R.E., and R.H. Moll. 1963. Genotype-environment interactions. p. 164–196. *In* W.D. Hanson and H.F. Robinson (ed.) Statistical genetics and plant breeding. Pub. 982. National Academy of Sciences-National Research Council, Washington, DC.

Comstock, R.E., and H.F. Robinson. 1952. Estimation of average dominance of genes. p. 494–518 *In* J.W. Gowen (ed.) Heterosis. Iowa State College Press, Ames, IA.

Falconer, D.S. 1983. Introduction to quantitative genetics. 2nd ed. Longman Group Limited, London and New York.

Hallauer, A.R., and J.B. Miranda. 1981. Quantitative genetics in maize breeding. Iowa State Univ. Press, Ames, IA.

Haunold, A. 1980. Hops. *In* W. Fehr and H. Hadley (ed.) Hybridization of crop plants. ASA, CSSA, and SSSA, Madison, WI.

Haunold, A. 1981. Hop production, breeding, and variety development in various countries. J. Am. Soc. Brew. Chem. 39:27–34.

Henderson, M.C., C.L. Miranda, J.F. Stevens, M.L. Deinzer, and D.R. Buhler. 2000. *In vitro* inhibition of human P450 enzymes by prenylated flavonoids from hops, *Humulus lupulus.* Xenobiotica 30(3): 235–251.

Henning, J.A., A. Haunold, and G. Nickerson. 1997a. Genetic parameter estimates for five traits in male hop accessions: A preliminary study. J. Am. Soc. Brew. Chem. 55(4):157–160.

Henning, J.A., A. Haunold, and G. Nickerson. 1997b. Estimates of heritability and genetic correlation for five traits in female hop accessions. J. Am. Soc. Brew. Chem. 55(4):161–165.

Keller, K.R., and S.T. Likens. 1955. Estimates of heritability in hop, *Humulus lupulus* L. Agron. J. 47:518–521.

Kempthorne, O. 1957. An introduction to quantitative genetics. Wiley Pub., New York.

Kinghorn, B., and S. Kinghorn. 2001. Pedigree viewer software [Online]. Available at www-personal.une.edu.au/~bkinghor/pedigree. htm (modified 28 Feb. 2005; verified 28 Mar. 2005). B. and S. Kinghorn, Armidale, NSW.

Miranda, C.L., J.F. Stephens, V. Ivanov, M. McCall, B. Frei, M.L. Deinzer, and D.R. Buhler. 1999. Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (*Humulus lupulus*) in human cancer cell lines. Food Chem. Toxicol. 37(4):271–285.

Miranda, C.L., J.F. Stephens, V. Ivanov, M. McCall, B. Frei, M.L. Deinzer, and D.R. Buhler. 2000. Antioxiddant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones. Agric. Food Chem. 48(9):3876–3884.

Neve, R.A. 1991. Hops. Chapman and Hall Publ., London, New York.

Roberts, D.D., W.E. Kronstadt, and A. Haunold. 1980. Genetic variability and association of maturity, yield, and quality characteristics of female hops. Crop Sci. 20:523–527.